REMARKS

Applicant acknowledges with appreciation the Examiner's August 23, 2006 withdrawal of the previously-issued rejections.

In the August 23, 2006 Office Action, the Examiner rejected claims 25 and 40-57 on the ground of nonstatutory obviousness-type double patenting "as being unpatentable over claims of U.S. Patent No. 7,063,838." As suggested by the Examiner, applicant is filing herewith a Terminal Disclaimer in which the terminal portion of the instant application is disclaimed to the extent it exceeds the full statutory term of U.S. Patent No. 7,063,838, thus obviating the last remaining rejection. Accordingly, applicant requests that this rejection be withdrawn and the claims allowed to issue.

In addition, applicant is amending the specification to recite the U.S. Patent Number of the parent application and to correct the following typographical error in paragraph [0062] (emphasis added):

The "1x" tissue sample was treated with collagenase 156 Mandel units/ml + elastase 0.125 mg/ml + trypsin inhibitor 038 mg/mg, The "2x" sample was treated with collagenase 312 Mandel units/ml + elastase 0.25 mg/ml + trypsin inhibitor 0.76 mg/ml. The "5x" sample was treated with collagenase 780 Mandel units/ml + elastase 0.625 mg/ml + trypsin inhibitor 1.9 mg/ml.

The recitation of the concentration of trypsin inhibitor in the "1x" sample as "038 mg/mg" was a typographical error. It is clear from the remainder of that paragraph that the concentration of trypsin inhibitor in the "1x" sample should have been recited as "**0.38 mg/ml**" which is one-half of the concentration (0.76 mg/ml) stated for the "2x" sample and one-fifth of the concentration (1.9 mg/ml) stated for the "5x" sample.

In seeking to correct this typographical error in the original specification, Applicant filed a Preliminary Amendment on September 30, 2005, but in that Preliminary Amendment inadvertently cited the figure given in the originally filed specification as "0.38" rather than "038" and omitted to correct "mg/mg" to "mg/ml". Accordingly, Applicant now submits this

Amendment in order to correct the typographical error in the originally filed specification from "038 mg/mg," to "0.38 mg/ml."

Finally, applicant sincerely thanks the Examiner for agreeing to consider and make of record the following references that are of record in the prosecution history of the parent application (now U.S. Patent No. 7,063,838):

Dobrin & Mrkvicka, *Cardiovas. Surg.*, 2(4): 484-488 (1994); and

Trubel et al., *Eur J. Vasc. Endovasc. Surg.*, 10: 415-423 (1995).

CONCLUSION

Applicant respectfully requests that the Examiner enter the amendments described herein and allow the claims to issue.

Applicant requests that the Terminal Disclaimer fee of $65.00 be charged to Fried, Frank, Harris, Shriver & Jacobson LLP Deposit Account No. 06-0920. Applicant believes that no additional fees are required. In the event that any fee is required, the Director is hereby authorized to charge any required fees to Fried, Frank, Harris, Shriver & Jacobson LLP Deposit Account No. 06-0920.

Respectfully submitted,

Date: September 29, 2006

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VASCULAR PAPERS

Failure of elastin or collagen as possible critical connective tissue alterations underlying aneurysmal dilatation

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Previous studies in the authors' laboratory have demonstrated that degradation of arterial elastin produces vessel dilatation, decreased vessel distensibility, and vessel elongation which can cause tortuosity. By contrast, degradation of collagen produces increased vessel distensibility and rupture. However, neither degradation of elastin nor of collagen produced the true gross enlargement characteristic of human aneurysms. The present study was performed to identify the connective tissue critical to aneurysm formation. Vessel dimensions were measured repeatedly in human arteries during progressive enzymatic degradation. Experiments were performed on six intact human common, external and internal iliac arteries, and two aneurysmal human common iliac arteries. The vessels were mounted *in-vitro* and subjected to pressure steps up to 200 mmHg while diameters were measured. Repeated pressure–diameter curves were obtained for up to 18 h during treatment with elastase or collagenase. Degradation of elastin produced moderate dilatation (6–10% at 100 mmHg) with decreased vessel distensibility; this occurred as the load was shifted to remaining collagen. Degradation of collagen produced greater dilatation (10–23% at 100 mmHg), increased distensibility, and vessel rupture. These findings suggest that the critical element in both the gross enlargement and rupture of aneurysms resides in collagen. They also suggest that, in vessels obtained from patients with a family history of aneurysms, defects should be sought in: (I) the structure of collagen; (II) increased susceptibility of collagen to degradation by endogenous mechanisms; (III) increased endogenous collagenolytic activity; or (IV) decreased inhibition of endogenous collagenolytic activities.

Keywords: aneurysms, elastin, collagen, connective tissue failure

Previous studies[1] have shown that dog and human vessels treated with elastase undergo dilatation but do not rupture, whereas vessels treated with collagenase dilate and promptly rupture. These data were interpreted as evidence that dilatation was due to failure of elastin, and rupture to failure of collagen. However, in most cases, neither treatment produced the gross dilatation which occurs with the development of human aneurysms. Tilson and co-workers[2] challenged the authors' view that failure of elastin is the critical element in human aneurysms. They may be correct because when vessels are treated experimentally with collagenase they rupture so rapidly that they cannot manifest the gradual enlargement characteristic of aneurysms in patients. In order to investigate the roles of elastin and collagen in human vessels, six intact human arteries and two human aneurysms treated with proteolytic enzymes were examined in a stepwise fashion. These vessels were subjected to repeated half-hourly or hourly assessment of vessel dimensions during the process of degradation in order to determine the degree of dilatation that occurred before rupture. The question of which connective tissue is most critical for the dilatation and rupture of aneurysms is important because it identifies which tissue in human aneurysms warrants study using the techniques of molecular biology.

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0967-2109/94/040484–05

Materials and methods

Six human intact internal, external, and common iliac arteries were excised from cadavers at autopsy. Two aneurysmal common iliac artery aneurysms also were obtained at autopsy. The lengths of the vessels were measured carefully before excision. Vessels were cannulated at both ends with polyethylene tubing, mounted in a tissue bath, and restored to *in situ* length. Each was filled and bathed with Krebs–Ringer solution buffered to pH 7.44. The tissue bath was maintained at 37°C. The lumen of the vessels was pressurized in 10 or 15-mmHg steps up to 25 mmHg, and then in 25-mmHg steps up to 150 mmHg. Vessel diameter was measured with a linear displacement transducer. Pressure was maintained at each level until the vessel exhibited a steady diameter. Reproducible pressure–diameter curves were obtained after four or five stepwise pressurization cycles. The fluid in the lumen was then removed and replaced with Krebs–Ringer solution containing 40 units/ml purified elastase (Worthington ESFF, Freehold, New Jersey, USA) or 300 units/ml purified collagenase (Worthington CLSPA). In most cases, the vessels were treated first with elastase and then with collagenase. In some cases the vessels were treated only with collagenase. To examine the progressive effect of these enzymes, each vessel was perfused at 10 mmHg for 30 to 60 min with the enzyme in the lumen. The lumen was then drained and refilled with Krebs–Ringer solution devoid of enzymes. The mechanical behavior of the vessel was then assessed by obtaining stepwise pressure–diameter curves. The plain Krebs–Ringer solution was removed and the solution containing the enzyme reintroduced into the lumen. This sequence of treatment followed by testing was repeated every 30 to 60 min for up to 18 h or until the vessel exhibited an unchanging diameter or underwent rupture.

Several additional vessels were treated with elastase or collagenase and examined histologically. The vessels were fixed in 10% buffered formaldehyde, embedded in paraffin, and sectioned into 6-μm-thick sections which were stained with Verhoff's elastic stain or Masson's trichrome stain for collagen. After treatment with elastase these vessels exhibited fractured or absent elastic lamellae; after treatment with collagenase they exhibited decreased density of staining with Masson's trichrome.

Results

Figures 1–5 use the following format to depict pressure–diameter relationships for individual arteries. The open circles depict data observed after four or five relaxation cycles to obtain reproducible pressure–diameter curves. The closed symbols describe the behaviour of the arteries during progressive treatment with elastase. Selected curves are shown to demonstrate the progressive changes that occurred during treatment with elastase. Curves obtained during treatment generally lie above those obtained for the relaxed vessels. None of the vessels treated with elastase ruptured. The uppermost curves with open symbols depict the behavior of the vessels during treatment with collagenase. Data recorded after treatment with collagenase are plotted until the vessel ruptured.

Non-aneurysmal arteries

Figure 1 presents data for a non-aneurysmal common iliac artery. The relaxed vessel (open circles) exhibited considerable distensibility at low pressures. When treated with elastase (closed symbols) the vessel progressively dilated and became less distensible. When treated with collagenase (open symbols) the vessel dilated further, became more distensible, and ruptured after 1.5 h.

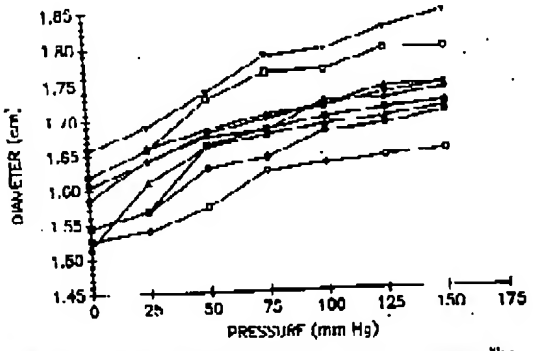

Figure 1 Pressure–diameter curves for a non-aneurysmal common iliac artery. Data are shown for the relaxed vessel (○), after treatment with elastase (●, 2 h; ▲, 5 h; ■, 6 h; ▼, 15 h; ◆, 18 h) and after treatment with collagenase (△, 0.5 h; □, 1 h; ▽, 1.5 h)

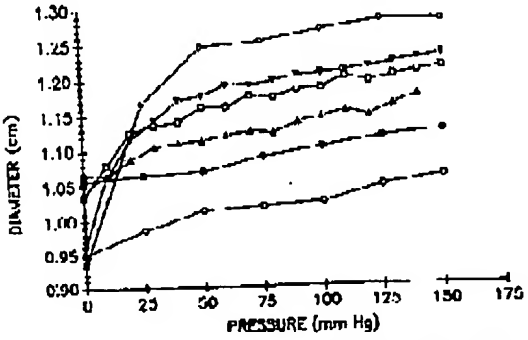

Figure 2 Pressure–diameter curves for a non-aneurysmal internal iliac artery. Data are shown for the relaxed vessel (○), after treatment with elastase (●) and after treatment with collagenase (△, 1 h; □, 2 h; ▽, 3 h; ◇, 4 h)

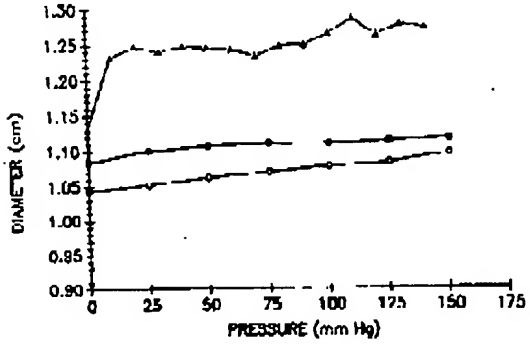

Figure 3 Pressure–diameter curves for a non-aneurysmal external iliac artery. Data are shown for the relaxed vessel (○), after treatment with elastase (●, 18 h) and after treatment with collagenase (Δ, 1 h)

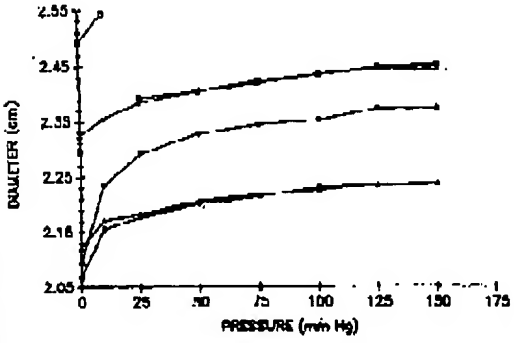

Figure 4 Pressure–diameter curves for an aneurysmal common iliac artery. Data shown for the relaxed vessel (Δ, ○), after treatment with elastase (■, ▲, 15 h; ●, 2 h) and after treatment with collagenase (□, 0.5 h)

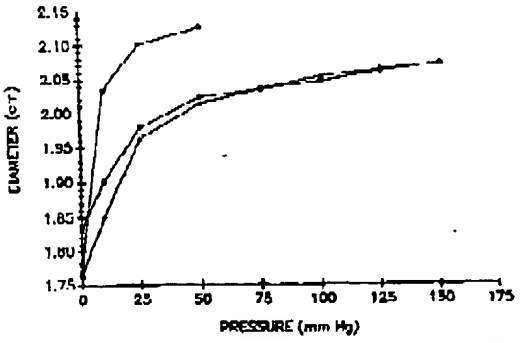

Figure 5 Pressure–diameter curves for an aneurysmal common iliac artery. Data are shown for the relaxed vessel (Δ, ○) and after treatment with collagenase (●, 2 h)

Figure 2 presents data for an internal iliac artery while relaxed (open circles), and after treatment with elastase (closed symbols). The vessel exhibited reproducible behavior at 10, 11 and 12 h after treatment with elastase. Also shown are pressure–diameter curves after treatment with collagenase with hourly pressure–diameter curves obtained up to 4 h. This vessel dilated markedly after treatment with collagenase and then ruptured.

Figure 3 presents data for an external iliac artery, with only moderate dilatation observed even after 18 h of elastase treatment. By contrast, the vessel underwent marked dilatation after only 1 h of collagenase treatment and ruptured after 1 h.

Aneurysmal arteries

Figure 4 presents pressure–diameter curves for an aneurysmal common iliac artery. Two curves are shown for the relaxed vessel. When treated with elastase the vessel dilated, showing reproducible pressure–diameter curves after 15 h. On treatment with collagenase, it dilated markedly, even at 0 mmHg, and ruptured when pressurized to 10 mmHg. As a result no data points could be obtained at pressures > 10 mmHg.

Figure 5 presents data for a second aneurysmal common iliac artery. This was relaxed (open symbols) and then treated with collagenase (closed symbols) without initial treatment with elastase. Treatment with collagenase caused marked dilatation and rupture at 50 mmHg, such that data could not be obtained at pressures > 50 mmHg.

In summary, during treatment with elastase, all vessels dilated, usually to a moderate degree. The dilatation occurring at 100 mmHg was 6 to 10%. In addition, most vessels exhibited some stiffening at low pressures as the distending load was shifted to collagen. None of the elastase-treated vessels ruptured. During gradual treatment with collagenase, all vessels dilated, most to a greater extent than they had after elastase treatment. After treatment with collagenase the dilatation occurring at 100 mmHg was 10 to 23%. The two aneurysmal arteries dilated profoundly during collagenase treatment and ruptured at pressures of 10 and 50 mmHg respectively. Therefore, they provided no data at 100 mmHg. All non-aneurysmal vessels also ruptured when treated with collagenase. Thus, even when studied in deliberate stepwise fashion, degradation of collagen produced rapid, extremely dramatic dilatation and rupture. These observations suggest that the intact vessel behaves like a compliant rubber tube (elastin) inside a slightly larger sleeve of a stiff protective steel net (collagen), and that is failure of collagen and not elastin which permits vessels to dilate and become aneurysmal. It may be concluded therefore that the interpretation by Tilson and co-workers[2] of the authors' earlier results of proteolytic degradation of elastin and collagen are correct.

Discussion

An underlying assumption of these experiments is that the enzymes used are relatively specific for their intended substrate, with little or no overlap of activity; this assumption is supported by experimental data. The collagenase used here, CLSPA (Worthington), has been found to have remarkably high specificity for collagen with negligible activity for elastin and other proteins such as casein[3]. In addition, histologic examination of vessels treated with elastase exhibited fractured or absent elastic lamellae, with no apparent reduction in Masson's trichrome staining of collagen. By contrast, vessels treated with collagenase showed decreased uptake of Masson's trichrome with continued uptake of Verhoff's elastic stain by the elastic lamellae. Also the mechanical responses after enzymatic treatment were very different. With elastase, all the vessels dilated to a stable diameter, at which they remained, and in no case ruptured. By contrast, after collagenase treatment all vessels dilated and then ruptured. Hence, it is reasonable to conclude that the actions of the enzymes were largely mutually exclusive.

The classic explanation for aneurysm formation is that they result from atherosclerotic degeneration of structural elements in the wall. This view is based on the observation that they often occur in conjunction with, or in proximity to, atherosclerotic lesions. Zarins and colleagues[4] have provided experimental evidence for this concept by feeding cynomolgus monkeys an atherogenic diet for 16 to 24 months; the animals then were fed an atherosclerosis-regression diet. Following regression of the disease, 13% of the animals developed arterial aneurysms, suggesting that the atherosclerotic degeneration of load-bearing elements in the wall may have caused the wall to fail mechanically.

Tilson and Stansel[5] have provided clinical/epidemiological evidence to suggest that many human aneurysms may not result from atherosclerosis, noting that patient age, male-to-female ratio, and clinical prognosis during follow-up are quite different in patients with atherosclerotic disease as compared with those with aneurysmal disease. Several studies have reported a familial predisposition to develop aneurysms[6–8]. Tilson and Seashore[6] studied the inheritance of more than 50 families with two or more relatives with aneurysms. Norrgard *et al.*[7] reported that 187 of 200 patients with aneurysms had relatives with similar lesions. Johansen and Koepsell[8] estimated that relatives of people with aneurysms have an 11.6 times increase in the risk of developing an aneurysm. Thus, there is evidence strongly suggestive of a genetic predisposition for aneurysm formation in the arteries of these patients. Tilson and Stansel[5] also suggested that atherosclerosis observed in the presence of aneurysms may be incidental, as most such patients are in their sixth or seventh decades, an age when many individuals in western society have atherosclerosis. Indeed, if atherosclerosis were the fundamental cause of most aneurysms then one might expect most patients with advanced atherosclerosis to develop aneurysms. This is not the case, the majority developing occlusive disease.

Inflammation also may play a role in aneurysm formation. Gertz *et al.*[9] produced experimental aneurysms by periarterial application of calcium chloride *in vivo*. Disruption of the elastic network in the wall was observed. The calcium–elastic tissue complex was the focus of an inflammatory, atherosclerotic reaction, and this was accompanied by the development of an aneurysm. Recently, Anidjar *et al.*[10] produced experimental aneurysms in rats *in vivo* by perfusing the infrarenal aorta with elastase, which led to aortic dilatation of 30% after 2 h of perfusion. Vessel caliber remained stable for several days but then dilated profoundly to three times the original dimension. This large secondary dilatation was accompanied by a marked influx of macrophages and active T cells. The present data suggest that these cells may have contributed to the degradation of collagen in the wall. Infusion of non-specific inflammatory agents also produced arterial aneurysms[10], but did so more gradually than when the vessels were treated with elastase. Pharmacological inhibition of the inflammatory processes to decrease the degree of dilatation has recently been shown (unpublished observations).

Both elastin and collagen are altered in the aneurysmal arterial wall. Several biochemical studies report that, when compared with normal arteries, the aneurysmal wall possesses decreased relative concentrations of elastin[11–14]. Zarins and co-workers[15] produced graded crush injuries in the thoracic aorta of pigs. The intact wall possesses about 75 elastic lamellae. When crush injury reduced the number of intact lamellae to less than 40, the vessels became aneurysmal. This corresponded to a mean rise in circumferential tension from 13.1×10^{-3} N/cm per lamellae in the intact vessel to 40.9×10^{-3} N/cm per lamellae in the crushed vessel. However, the crush may also have damaged collagen fibres which do not appear histologically as identifiable lamellae.

Collagen may be altered in arterial aneurysms. Rizzo *et al.*[11] and Menashi and colleagues[16] reported increased relative concentrations of collagen in aneurysms, presumably the result of preferential loss of elastin. Powell and Greenhalgh[17] reported decreased type III collagen in aneurysms, although Rizzo *et al.*[11] and Menashi *et al.*[16] could not confirm this observation. McGee *et al.*[18] reported increased levels of type I and type III procollagen message levels in both human abdominal aortic aneurysms and human aortas with occlusive disease, as compared with undiseased aortas, but there was no difference in procollagen message levels between the two groups of diseased vessels. This suggests that both diseases predispose to increased synthesis of collagen; however, both groups of diseased aortas were from elderly patients whereas the normal vessels were from younger patients.

The present stepwise experiments suggest that collagen is the critical wall element in both the dilatation and the rupture of aneurysms. From past investigations[1,19,20] as well as from the present experimental findings (*Figures 1–5*), the mechanical roles of elastin and collagen in the pathophysiology of aneurysms may be summarized: (i) failure of elastin permits vessels to dilate to a moderate extent; (ii) failure of elastin also permits vessel lengthening and the development of tortuosity; (iii) failure of collagen permits vessels to undergo gross aneurysmal dilatation with a small amount of lengthening; (iv) recruitment of previously non-loaded collagen fibers and a change in geometry from a cylinder to a 'sphere' stabilizes the aneurysmal wall, thereby preventing it from rupturing immediately; (v) the thrombus lining the aneurysm contributes little to wall stability; and (vi) continued failure of collagen leads to vessel rupture.

It may then be asked why the connective tissues in the vessel wall fail. Kontusaari *et al.*[21] described a genetic defect in one member of a family in which there were large numbers of aneurysms. The defect was that glycine was substituted for arginine in type III collagen, although this observation remains to be replicated in the tissues of other patients. Nonetheless, even if there is a genetically determined tendency to form aneurysms, why do these lesions not manifest in most patients until they are in their sixth or seventh decade? The answer to this question may lie in degradation of elastin with age. Aged vessels often show histologic evidence of reduced numbers of elastic fibers. Similarly, pressure–volume curves of segments of human thoracic aorta show that, with age, the arteries gradually dilate and become stiffer[22]. This suggests that with age, elastin gradually fails permitting dilatation to occur with shifting of the load from elastin to previously non-loaded collagen. This is similar to what has been observed when elastin is degraded experimentally using proteolytic enzymes[1]. If the recruited collagen fibers are sound, then they will support the artery and in so doing contribute their stiffness to the wall. However, if the collagen fibers are mechanically defective, are abnormally susceptible to enzymatic degradation, attract excessive numbers of inflammatory cells which release proteolytic enzymes, or lack normal levels of tissue inhibitors of metalloproteases that protect against endogenous proteolytic degradation[2,3], then the collagen in the wall will be predisposed to degradation. As this proceeds, the vessel will be unable to withstand the distending force resulting from luminal pressure. This will permit progressive aneurysmal dilatation, and a further increase in circumferential distending force, eventually leading to vessel rupture.

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Paper accepted 26 August 1993

Eur J Vasc Endovasc Surg 10, 415–423 (1995)

Compliance Mismatch and Formation of Distal Anastomotic Intimal Hyperplasia in Externally Stiffened and Lumen-adapted Venous Grafts*

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Objective: Compliance and formation of distal anastomotic intimal hyperplasia (DAIH) were investigated in externally stiffened venous grafts of varying calibers.

Methods: 36 femoropopliteal reconstructions were performed in 18 sheep. The autologous venous grafts were inserted into tubes made of Dacron mesh to achieve compliance-mismatch and lumen adaptation. Compliance was measured by echo-tracked ultrasonography and profiles of DAIH were generated from histologic sections harvested after 8.3 months.

Main results: The external mesh tube significantly lowered the local compliance of graft and host artery. DAIH appeared extensively in those groups where mesh tube constricted venous grafts met untreated host arteries ($p < 0.001$). No differences in compliance and DAIH formation were observed when grafts with large and adapted diameters were compared.

Conclusions: For prevention of DAIH the distal venous graft diameter is not important, while the local compliance of an autologous vein is a predictive factor for DAIH formation and thus long-term patency.

Key Words: Compliance mismatch; Intimal hyperplasia; Distal anastomosis; Autologous grafts; Adaptation of venous graft lumen.

Introduction

Progression of intimal hyperplasia at distal end-to-side anastomoses remains a major cause of late bypass graft failure.[1–3] Mitogenic factors[4,5] and local platelet activation,[6–8] unphysiological flow patterns[9] and mechanical factors[10] have been implicated in the pathogenesis of distal anastomotic intimal hyperplasia (DAIH). Among the mechanical factors the mismatch in elastic properties between bypass graft and host artery has recently been correlated with DAIH in experiments and clinical practice.[1,11–13] Most of the studies dealing with compliance mismatch and DAIH formation investigated various prosthetic graft materials of different elasticity. However, these results may have been influenced by the prosthetic graft material itself. None of these studies investigated the effects of stiffening venous bypass graft materials, as by external reinforcement, on DAIH formation.

In our study, DAIH formation was investigated in distal end-to-side anastomoses of autologous venous grafts and host arteries with different compliances. Compliance reduction was achieved by external constriction of the vessels with a Dacron mesh tube. External Dacron mesh constriction of autologous veins has been reported to enable the use of dilated and varicose veins for coronary and peripheral vascular procedures and to match the venous graft lumen to the diameter of grafted arteries in coronary surgery.[19–22] The adaption of the bypass graft lumen to the host artery diameter has been reported to increase flow velocity and shear rate, which in turn has been inversely correlated to platelet activation[23] and formation of DAIH.[24] Besides the influence of compliance mismatch we studied the influence of different bypass graft calibers on the formation of DAIH.

*Presented at the 8th annual meeting of the European Society for Vascular Surgery, Berlin, Germany (September 1994).

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1078–5884/95/080415 + 09 $13.00/0

Table 1. Group schedule of bypass graft and host artery reconstructions and their cross diameters (in mm)

Group	Bypass graft	Host artery	Graft diameter	Host artery diameter
1	Natural vein	Natural artery	4.5 (±1.04)	4.14 (±0.49)
2	Mesh constricted vein	Natural artery	8.0 (±0)	4.4 (±0.42)
3	Mesh-constricted vein	Mesh-constricted artery	8.0 (±0)	4.13 (±0.8)
4	Natural lumen-adapted vein	Natural artery	4.29 (±0.49)	4.8 (±0.84)
5	Mesh-constricted lumen-adapted vein	Natural artery	4.0 (±0)	4.5 (±0.76)
6	Mesh-constricted lumen adapted vein	Mesh constricted artery	4.0 (±0)	4.33 (±0.52)

Materials and Methods

According to the Austrian law for animal experiments and after permission by the University Ethics Commission, 36 femoropopliteal bypasses were implanted in 18 sheep (body weight 62–71 kg). Under general anesthesia the femoral and popliteal arteries of both sides were dissected free, and the original superficial femoral arteries ligated. The reversed deep femoral vein was used as graft material in all operations. 2500 units of heparin were administered intravenously prior to arterial clamping on each side. All bypass graft anastomoses were sewn with 7/0 Prolene in a running stitch-technique.

Reconstructions were divided into six groups (Table 1, Fig. 1): In groups 1 and 4 native venous grafts without any external reinforcement were implanted. In groups 2,3,5 and 6 the venous grafts were inserted into tubes made of Dacron mesh fabric (Meadox Lars mesh, Oakland, NJ, U.S.A.) prior to implantation. These tubes were sewn over a mandril (diameters of 8mm [groups 2 and 3] and 4mm [groups 5 and 6]) with 4/0 silk in a locking stitch technique and were included into the suture lines of the proximal and distal anastomoses. In groups 3 and 6, 2cm of the adjacent host arteries were also supported by external mesh tubes, which were wrapped around the host artery and fixed with 7/0 Prolene sutures after distal graft anastomosis. In groups 1–3 the diameters of the grafts were not narrowed, they remained approximately twice as big as the host artery diameters (Table 1). In group 1 the venous grafts remained natural, in groups 2 and 3 mesh tubes sewn over a mandril of 8mm were used for external support. In groups 4–6 the bypass graft diameters were adapted to the host artery diameter of approximately 4mm (Table 1). In group 4 the natural venous graft lumen was adapted to the host arterial lumen by transvere single stitches controlled by a caliper. In groups 5 and 6 the graft lumen was adapted by mesh tubes sewn over a 4mm mandril. Each group comprised six bypass procedures and the group distribution to each animal's leg was random.

Graft and host artery diameters were measured with an electronic sliding caliper (Mitutoyo™ Digimatic, Tokyo, Japan). Blood flow was measured electromagnetically (Hellige™, Freiburg, Germany) in the native femoral artery prior to its ligation and in the

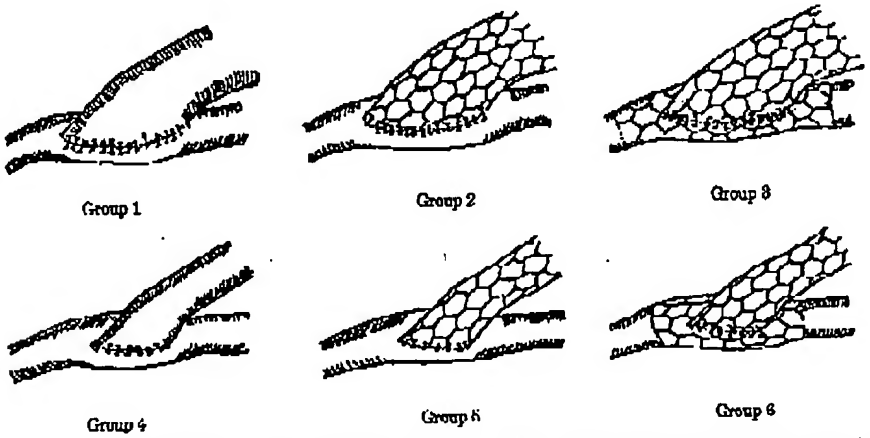

Fig. 1. Reconstructions were divided into 6 groups consisting of native and mesh-constricted venous grafts and host arteries with natural and adapted graft lumens.

bypass grafts 10 min after implantation. The compliances of the different grafts, the distal anastomotic suture regions and of the host arteries close to the distal graft anastomosis were evaluated sonographically. Three pairs of opposite crystalloid sensors (Vessel diameter CVD 2000, Sonotek Corp., San Diego, CA, U.S.A.) were temporarily fixed to the external vessel surface at the same cross sections (Fig. 2: sections A,B [directly at the suture line] and D). Pulsatile changes on the diameters of each pair of crystalloids were measured based on local wall elasticities. At the same time local arterial blood pressures were recorded invasively. From these data the compliances of the different grafts, the distal anastomotic suture regions and of the host arteries were calculated according to the equation:

wall compliance* = $\Delta d / [d \times (p_{sys} - p_{dia})]$

(d = diameter measured with crystals, p = local arterial blood pressure)

*given in units of percent change in diameter / mmHg $\times 10^{-3}$

Local flow velocity profiles were measured with a paravascular ultrasound doppler device (Dr. Hartley, Houston, U.S.A.) with computerised post-processing using an ultrasound scanning frequency fo 20 MHz.[25] In particular, the sagittal flow profiles at the anastomosis were obtained according to the method previously described.[20] At the end of each procedure completion angiograms of each reconstruction were performed. After surgery the animals were kept under natural farming conditions without any medication until the final follow-up investigation, which was performed after a mean of 8.16 months.

At follow-up, the bypass reconstructions of both legs were again dissected free under general anesthesia. Blood flow in each bypass graft was again measured electromagnetically. The compliances of each graft, of the distal anastomotic suture regions and of the host arteries were recorded in the same way and at the same locations as before. The animals were killed by i.v injection of a potassium solution. The bypass grafts including the distal anastomotic regions and 2cm of the adjacent host arteries were fixed with 3% glutaraldehyde for 20 min under pressure similar to normal arterial pressure (mean of 100 mmHg). The samples were then explanted and prepared for histological examination. Cross sections of each specimen were taken at five constant locations (Fig. 2). All

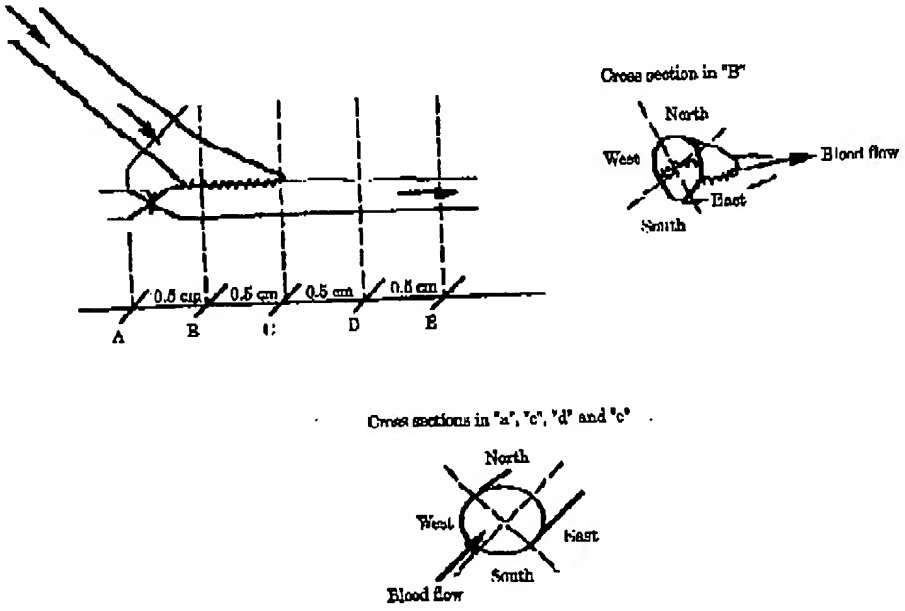

Fig. 2. Cross sections of the distal bypass anastomosis for histological examination of DAH.

Table 2. Electromagnetic blood flow measurements (in ml/min): Comparison of the groups (Mann-Whitney U-test)

Group	Native artery prior to ligation	Bypass graft intraoperatively	Bypass graft during follow-up investigation
1	111.75 (±19.9)	95.29 (±46.9)	108.75 (±14.8)
2	145.0 (±56.8)	128.75 (±55.1)	137.0 (±23.4)
3	191.67 (±29.5)	156.67 (±29.4)	113.0 (±10.9)
4	222.5 (±31.2)	122.5 (±40.9)	176.67 (±58.6)
5	215.0 (±83.2)	176.25 (±65.5)	153.33 (±57.8)
6	195.0 (±19.3)	122.0 (±81.4)	127.5 (±56.5)
	p=NS	p<NS	p=NS

specimens were embedded and coloured by Elastic-Van Gieson's stain. The histomorphological examination of the blinded specimen included identification and localisation of intimal hyperplasia and morphometrical measurements of DAIH thickness at each cross section.

All data were entered into a computer-based spreadsheet (Excel™, Microsoft Inc., CA, U.S.A.). Statistical analysis of selected groups was performed using the Mann-Whitney U-test (Program Package SPSS Inc., Chicago, Ill., U.S.A.).

Results

The average length of the implanted grafts was 9.21cm (±1.43). In the groups with a calibre mismatch between graft and host artery (groups 1–3) the diameter ratio between grafts and host arteries was 1.99 (±0.31):1, in the groups with the same calibre (groups 4–6) the ratio was 0.9 (±0.15):1 (Table 1). The blood flow was comparable between the groups: the highest flow rates were recorded in the native arteries prior to ligation. In groups 1,2,4 and 6 the lowest flow rates were observed intraoperatively. In groups 3 and 5 the lowest flow rates were observed during the follow-up investigation. Differences in blood flow between the groups were not significant (Table 2). Table 3 shows the compliances of graft wall, anastomotic region and host artery in each group. Apart from the host arteries in group 1 local compliances were similar in the primary procedure (OP) and the follow-up (FU). Comparison of the groups with calibre mismatch (groups 1–3) and the groups with adapted graft lumen (groups 4–6) did not show significant differences in local compliance (Table 3). Compliance was found to be significantly lowered by external constriction with a Dacron mesh tube (Table 4). This was seen when comparing the groups with natural venous grafts (groups 1 and 4) to the groups with mesh-constricted grafts (groups 2 and 5 and groups 3 and 6, respectively) and when comparing the groups with a natural host artery (groups 1 and 4 and groups 2 and 5, respectively) to the groups with a mesh-constricted host artery (groups 3 and 6). No differences were seen in the compliances of the anastomotic regions (Table 4).

Table 5 shows the extent and distribution of DAIH areas in the cross sections of each group. Intimal thickening developed at two distinct and separate sites: extensive formation of DAIH occurred at the suture lines (Fig. 2: sections B-"east" and "west" (see also Fig. 3) and section C-"north"), whereas moderate DAIH was observed on the floor of the artery (Fig. 2: section B-"south") and behind the anastomotic lip (Fig 2: section D-"north", Fig. 4).

DAIH formation was found to be significantly larger when a compliance mismatch between bypass graft and host artery had been induced (Table 6). It

Table 3. Compliances of grafts and host arteries (units given in 10^{-4}) during primary procedure (OP) and follow-up investigation (FU) Comparison between mismatched and matched bypass graft calibre (Mann-Whitney U-test)

	Native venous grafts		Mesh constricted grafts		Mesh constricted grafts and host arteries	
Localisation	Group 1 large diameter	Group 4 adapted diameter	Group 3 large diameter	Group 5 adapted diameter	Group 3 large diameter	Group 6 adapted diameter
Graft (OP)	159.52 (±35.4)	196.81 (±26.2)	66.84 (±19.3)	55.89 (±17.6)	46.93 (±26.4)	42.77 (±10.2)
Graft (FU)	126.39 (±29.1)	107.7 (±13.58)	56.34 (±17.9)	51.14 (±19.9)	43.75 (±15.9)	57.93 (±27.8)
Anastomosis (OP)	97.38 (±79.3)	85.0 (±18.31)	68.2 (±37.5)	53.18 (±14.0)	40.6 (±23.7)	39.49 (±21.9)
Anastomosis (FU)	62.8 (±20.5)	47.56 (±18.51)	53.72 (±21.6)	51.58 (±15.2)	38.8 (±17.5)	61.96 (±31.0)
Host artery (OP)	281.61 (±91.0)	359.29 (±148.9)	370.7 (±148.4)	203.1 (±120.4)	76.31 (±30.6)	52.71 (±12.7)
Host artery (FU)	385.2 (±98.0)*	269.76 (±37.16)	355.1 (±140.2)	220.26 (±37.2)	54.55 (±24.5)	47.26 (±21.5)

OP vs. FU in group 1: *p=0.015.

Table 4. Compliances of grafts and host arteries (units given in 10^{-6}) during primary procedure (OP) and follow-up investigation (FU). Comparison between natural and mesh-constricted vessels (Mann-Whitney U-test)

Localisation	Groups 1 and 4 natural vein grafts/ natural host arteries	Groups 2 and 5 mesh tube grafts/ natural host arteries	Groups 3 and 6 mesh tube grafts/ mesh tube host arteries
Graft (OP)	172.5 (±48.0)	63.1 (±23.4)*	46.6 (±22.1)†‡
Graft (FU)	147.9 (±23.2)	54.6 (±19.6)**	50.8 (±22)‡†
Anastomosis (OP)	53.4 (±11.2)	59.0 (±36.0)	44.7 (±22.9)
Anastomosis (FU)	58.1 (±38.7)	51.2 (±17.7)	49.1 (±31.5)
Host artery (OP)	204.0 (±114.1)	331.9 (±124.9)	64.1 (±25.4)†‡‡
Host artery (FU)	311.1 (±83.8)	288.1 (±115.1)	51.2 (±22.3)†‡‡

Groups 1 and 4 vs. groups 2 and 5: *p=0.003, **p=0.006.
Groups 1 and 4 vs. groups 3 and 6: †p=0.0015, ††p=0.002, †††p=0.003.
Groups 2 and 5 vs. groups 3 and 6: ‡p=0.001, ‡‡p=0.012.

was most pronounced in the groups with mesh-constricted grafts and natural host arteries (groups 2 and 5) as compared to the groups with native bypasses (groups 1 and 4) and the groups with mesh constriction over the graft and the adjacent host artery (groups 3 and 6). No differences were observed between all natural (groups 1 and 4) and all mesh-constricted (groups 3 and 6) reconstructions. Statistical comparison of formation and extent of DAIH in the groups with a calibre mismatch (groups 1–3) and the groups with adapted graft lumen (groups 4–6) showed no differences in DAIH formation (Table 7).

Areas of flow reversal near the toe of the distal graft anastomosis were found in 13 grafts by means of 8-channel Doppler measurements. Their occurrence could be correlated with the overall incidence of hyperplasia but not with the hyperplasia in this particular region. The detailed results of these measurements are given elsewhere.[26]

Discussion

The concept of Baird and Abbott[12] that compliance mismatch between bypass graft and artery plays an important role in the development of anastomotic intimal hyperplasia has been valid since 1976 although the pathogenesis of DAIH is now considered to be more complex.[9–10, 13, 27–29] Graft compliance has been recently correlated with long-term patency rates[1,12] and DAIH remains a problem in synthetic vascular prostheses.[30,31] Most experimental studies directly examining the relationship between compliance mismatch and DAIH have dealt with prosthetic graft materials of different elasticity.[15–18] In all these studies, the positive correlation between compliance mismatch and DAIH formation have been confirmed.

Intimal hyperplasia refers to the proliferation of subintimal smooth muscle cells that migrate through defects in the internal elastic lamina and continue to proliferate and secrete matrix proteins, thus leading to intimal thickening and intimal hyperplasia. Intimal thickening can also result from the sequelae of mural thrombus organisation. In an advanced stage it can be very difficult to differentiate a well organised luminal thrombus from original intimal hyperplasia.[30,32–38] Based on this fact, Hong-De Wu *et al*.[8] postulated that DAIH is just a late result of well organised local thrombosis at the anastomotic site thus contradicting the importance of compliance mismatch for DAIH formation. Their assumption was based on experiments where the authors did not observe significant

Table 5. Extent and localisation of distal anastomotic intimal hyperplasia = DAIH (mean values in mm)

Cross section	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
A (distal graft)	28.17 (±4.66)	13.25 (±1.25)	20.47 (±9.41)	26.86 (±1.55)	14.48 (±4.88)	24.7 (±10.01)
B (0.5 cm before tip)	58.9 (±45.53)	109.14 (±41.30)	94.38 (±50.5)	105.32 (±28.69)	176.17 (±106.38)	110.41 (±37.79)
C (anastomotic tip)	27.29 (±12.18)	80.45 (±54.43)	12.54 (±18.77)	16.63 (±12.6)	44.17 (25.18)	14.5 (±17.9)
D (0.5 cm behind tip)	6.72 (±7.81)	47.36 (±34.5)	8.75 (±19.37)	<5 (±0)	30.18 (±22.06)	0.92 (+2.05)
E (1 cm behind tip)	7.63 (±1.25)	6.71 (±4.89)	<5 (±0)	<5 (±0)	6.69 (±7.76)	<5 (±0)
North (top)	5.75 (±5.75)	5.33 (±5.75)	3.07 (+5.34)	<5 (±0)	22.1 (±11.79)	4.5 (±10.06)
East (right wall)	25.4 (±11.29)	76.69 (±18.40)	39.72 (+13.19)	32.94 (±9.39)	85.18 (±48.68)	42.72 (±23.04)
South (bottom)	17.8 (±18.72)	42.69 (±17.77)	3.98 (±4.56)	24.69 (±27.52)	34.63 (±25.8)	5.12 (±3.53)
West (left wall)	28.48 (±13.87)	66.21 (±23.97)	45.77 (±19.51)	39.93 (±7.39)	63.92 (+28.16)	54.72 (±22.01)
Total (mean A–F)	18.11 (±10.29)	46.73 (±13.51)	23.13 (±6.17)	24.39 (±6.59)	51.44 (±28.50)	26.77 (±11.07)

Fig. 3. Cross section in the distal bypass anastomosis (= section "B") of a mesh tube constricted venous graft (= "g"; large diameter = group 2) with an untreated host artery (= "a"); h = intimal hyperplasia in the suture line region, * = Dacron mesh fibres

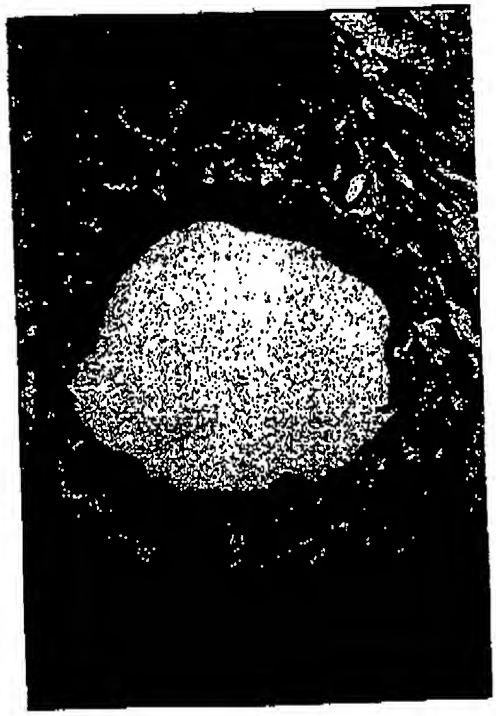

Fig. 4. Cross section in a host artery (1 cm behind the anastomotic tip (= section "D") of a mesh tube constricted venous graft (adapted diameter - group 5) with an untreated host artery: location of DAIH in section "north"

differences in DAIH formation between compliant and non-compliant Dacron grafts in dogs with a low thrombogenic potential. We aimed to exclude thrombogenic or any other influences from prosthetic graft surfaces in our trial set-up. We investigated the effects of compliance-mismatch and DAIH formation on distal end-to-side anastomoses using autologous veins where the compliances of the bypass grafts and host arteries were lowered by external constriction of the vessels with Dacron mesh tubes.

Another aim of our study was to elucidate if an adaption of bypass graft lumen to the host artery diameter would further influence DAIH formation. The influence of bypass graft diameter on DAIH has been demonstrated by Binns et al.[24] in differently sized PTFE grafts. DAIH was observed lowest in grafts with diameters equal to the host arteries and was found to

Table 6. Formation of distal anastomotic intimal hyperplasia (DAIH): Comparison of natural and mesh-constricted vessels (Mann Whitney U-test)

	Groups 1 and 4 natural vein grafts/ natural host arteries	Groups 2 and 5 mesh tube grafts/ natural host arteries	Groups 3 and 6 mesh tube grafts/ mesh tube host arteries
DAIH (mean in µm)	20.8 ± 0.96*	49.62 ± 22.9†	24.90 ± 8.66‡

Groups 1 and 4 vs. groups 2 and 5: *$p=0.001$.
Groups 1 and 4 vs. groups 3 and 6: NS
Groups 2 and 5 vs. groups 3 and 6: †$p=0.003$.

Table 7. Formation of distal anastomotic intimal hyperplasia (DAIH): Comparison of mismatched and matched bypass graft calibers (Mann-Whitney U-test)

Caliber mismatch	DAIH (μm)	vs. Lumen adaption	DAIH (μm)	
Group 1 (nat. graft)	18.11 (±3.29)	Group 4 (nat. graft)	24.39 (±6.95)	NS
Group 2 (mesh graft)	46.73 (±18.51)	Group 5 (mesh graft)	51.46 (±18.53)	NS
Group 3 (all mesh)	23.33 (±6.17)	Group 6 (all mesh)	26.77 (±6.07)	NS
Groups 1, 2, 3	29.39 (±10.7)	Groups 4, 5, 6	34.4 (±14.85)	NS

be significantly higher in grafts with greater calibres. All grafts with a calibre smaller than the host artery failed early due to graft thrombosis and could not be evaluated for intimal hyperplasia. Binns reported an inversive correlation between DAIH occurrence and the flow velocity and local shear rate. In addition to mechanical mismatch, a wide variety of hemodynamic factors such as high and low flow[30-41] and high and low wall shear stress[42,43] have been implicated in intimal hyperplasia formation by causing local endothelial injury.[10]

At rest, the flow rate in a graft is determined primarily by downstream peripheral resistance in the native host arterial tree, and not by the diameter of the bypass graft used.[44] In our model, with comparable distal arterial run-off, we expected similar bypass graft flows. In this way we hoped to influence the flow pattern and the local shear stress by variation of the bypass graft diameter, as displayed in computer simulations by Perktold *et al.*[45,46] Compliance was significantly lowered in our study by external constriction with a Dacron mesh tube while it was not influenced by adaption of the bypass graft calibre to the diameter of the recipient artery. DAIH formation and extent were found to be significantly higher in the groups with a compliance mismatch between graft and artery in comparison to the isocompliant groups. These results are comparable to most of the studies dealing with compliance mismatch and DAIH formation in prosthetic grafts and we conclude that the mechanisms increasing DAIH in non-compliant autologous graft materials must be similar to those in prosthetic grafts. An assessment of abnormal wall thickening in autologous veins prior to implantation as arterial bypass grafts would therefore seem to be important. This has been shown by Davies *et al.* who demonstrated a significant reduction in the compliance of long saphenous veins prior to implantation when areas of intimal hyperplasia and venous muscle hypertrophy were present.[47] The relationship between lowered venous graft compliance and the consecutive development of local bypass graft stenosis was also highly significant in Davies's study. Clinically, the importance of local venous graft compliance was confirmed by Scott *et al.*,[48] who suggested that veins with existing areas of intimal hyperplasia may be more likely to undergo graft stenosis.

DAIH in our specimens occurred extensively at the suture lines, whereas moderate intimal thickening was observed on the floor of the artery and behind the anastomotic tip. Similar DAIH localisation and distribution has been reported by Sottiurai *et al.*[49] in thrombosed prosthetic grafts in humans and by Bassiouny *et al.*[9] in experimental PTFE grafts. Bassiouny was also able to reveal complex secondary flow patterns mainly in the vicinity of the suture line and stated that these flow patterns interacted with biomechanical and humoral factors to modulate intimal thickening primarily on the suture line.[9]

In contrast to the results of Binns *et al.*[24] and other studies dealing with various graft diameters in artificial grafts, we did not observe relevant differences in DAIH according to diameter mismatch. With the 8-channel flow velocity meter we were able to identify areas of temporary flow reversal in the anastomotic tip within the cardiac cycle, which have been predicted by theoretical studies.[45,46] We were able to correlate such recirculations with overall (NS) but not with local intimal hyperplasia.[26] Our measurements of the anastomotic flow profiles could not further elucidate constant differences in the flow patterns between mismatched and lumen adapted groups.

In conclusion, mismatch in compliance between an autologous venous graft and the host artery may play an important role in the development of DAIH. For prevention of DAIH, the distal venous graft diameter is less important, while the local compliance of an autologous vein is a predictive factor for DAIH formation and thus for long-term vein graft patency.

Acknowledgements

This study was supported by the Jubilaeumsfonds der Oesterreichischen Nationalbank (Grant Nr. 3788). The authors wish to thank the staff of the Centre for Biomedical Research for their cooperation and assistance.

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Accepted 10 February 1995